

Claims

1. A method for detecting the presence of biofilm-forming microorganisms in a paper or board making process for determining the need of an anti-biofilm agent in the process, **characterized** by the steps comprising:
 - 5 (a) subjecting a sampler device in the process line for a period of time to enable said microorganisms to form a biofilm *in situ* in said process on the surface of the sampler,
 - (b) optionally treating the surface of the sampler with said formed biofilm thereon in a solution of a test anti-biofilm agent for a period of time, then
 - 10 (c) contacting the surface of the sampler with said biofilm thereon with a liquid growth medium in a recession of a culturing device for a period of time,
 - (d) removing the growth solution and the surface of the sampler from the recession of said device and detecting qualitatively and/or quantitatively the presence or absence of a biofilm adhered on the walls of the recession.
- 15 2. The method according to claim 1 or 2, **characterized** after the biofilm formation said surface of the sampler is (b) treated with the solution of the test anti-biofilm agent for the selection of the most efficient anti-biofilm agent.
3. The method according to any of the preceding claims, **characterized** in that
 - (a) subjecting a sampler device in the process line for a period of 12 h to 3 d,
 - 20 (b) effecting the optional treatment step with the solution of a test anti-biofilm agent for a period of e.g. 10 minutes to 4 hours, preferably for 1-2 h, between the ambient temperature and 65°C, preferably at the temperature close to the process temperature of the sampling site of the process line, such as at 40-60°C, then
 - 25 (c) effecting the culturing step, preferably with shaking, in a liquid growth medium in a recession of a culturing device for a period of e.g. for 8-48 h, preferably for 8-24 h, at the temperature between the ambient temperature and 65°C, e.g. at 35-65°C, preferably close to the process temperature of the sampling site of the process line, such as at 40-60°C.

4. The method according to any of the preceding claims, **characterized** in that (b) the treatment is effected in a treatment device provided with a recession which is filled with a solution comprising the test anti-biofilm agent and a liquid growth medium, sterilized water and/or process water by immersing said surface of the
5 sampler in said solution.
5. The method according to any of the preceding claims, **characterized** in that the step (c) is effected in a culturing device provided with a recession which is filled with the liquid growth medium by immersing said surface of the sampler in said solution.
- 10 6. The method according to any of the preceding claims, **characterized** in that (d) the sampler surface and the growth solution is removed from the recession of the culturing device, the recession is optionally washed and any biofilm-forming microorganisms adhered on the walls of the recession are stained and the presence and/or intensity of the color formation in the recession is detected
15 qualitatively or quantitatively.
7. The method according to any of the preceding claims, **characterized** in that (a) the sampler device comprises a plurality of elongated protrusions connected to a support, whereby, when brought in the process, the biofilm is formed on the surface of the protrusions.
- 20 8. The method according to claim 8, **characterized** in that (b) the treatment device is provided with a plurality of recessions containing a solution comprising one or more test anti-biofilm agents in one or more concentrations, one test anti-biofilm agent at one concentration in each recession, and said solution without any test anti-biofilm agent as a reference, and that the protrusions of said sampler
25 removed from the process line are immersed in said solution in the recessions, one protrusion in each recession.
9. The method according to claim 8 or 9, **characterized** in that (c) the culturing device comprises a plurality of recessions containing the liquid growth medium, and that the protrusions of said sampler, optionally treated in step (b), are
30 immersed in said growth solution in the recessions of the culturing device, one protrusion in each recession.
10. The method according to any of the preceding claims, **characterized** in that the sampler device comprises a plurality of pins or pegs arranged in rows and fixed from one end on a support plate and the treatment device of the optional step

(b) and the culturing device of the step (c) are multi-well plates provided with a plurality of wells arranged in rows and adapted for receiving one protruding pin in one well so that each pin of the sampler device sits in each well of the plate of the treatment and culturing device.

- 5 11. An assembly kit for detecting the presence of biofilm-forming microorganisms according to the method of claim 1 in a paper-making or board-making process, and for determining the need of an anti-biofilm agent in the process, and optionally for the selection of the most efficient anti-biofilm agent, comprising at least a combination of
- 10 (i) a sampler device comprising a plurality of elongated protrusions connected to a support for enabling said microorganisms to form a biofilm *in situ* in said process on the surface of the sampler,
- (ii) an optional treatment device comprising a plate provided with a plurality of recessions arranged to receive one protrusion of the sampler device in each
- 15 recession thereof,
- (iii) a culturing device comprising a plate provided with a plurality of recessions arranged to receive one protrusion of the sampler device in each recession thereof,
- (iv) a shaker for shaking the treatment and/or the culturing device, and
- 20 (v) optionally a detector for detecting the presence or absence of any stained microbes adhered in the recessions of the culturing device,
- (vi) reagents comprising
- (a) one or more test anti-biofilm agents suitable for the paper industry, preferably in one or more dilutions,
- 25 (b) a liquid growth medium,
- (c) staining agent, and
- (d) optionally a washing solution.

12. The assembly kit according to claim 12, **characterized** in that it comprises (i) a sampler provided with a plurality of pins in rows fixed from one end on a lid and
- 30 (ii) the treatment device and (iii) the culturing device, which are both multi-well

plates provided with a plurality of wells in rows, whereby the pins of the sampler sit in the wells of the treatment and of the culturing plate, one pin in each well, when the sampler lid is placed on the treatment or the culturing plate.

5 13. The assembly kit according to claim 12 or 13, **characterized** in that (ii) the wells of the treatment plate are filled with a solution of one or more test anti-biofilm agents, at one or more concentrations, in a liquid growth medium or sterilized water, one test anti-biofilm agent in each well, and that at least one well is filled with a growth medium or sterilized water alone, and (iii) the wells of the culturing plate are filled with a liquid growth medium, and that the wells are sealed off with a
10 removable cover.

14. The use of the detection method of any of claims 1-11 or of the assembly kit of any of claims 12-14 for monitoring the paper or board-making process to determine the need of an anti-biofilm agent in the process, and for the selection of the most efficient anti-biofilm agent.

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